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Macrophage Functions after Exposure to Nonfibrous Mineral Dusts

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The effects of the standard dusts, electrocorundum, and Dorentruper quartz (DQ_{12}), as well as mine dusts have been tested in guinea pig lung macrophage cultures. The parameters compared were: release of lactic dehydrogenase for demonstration of plasma membrane permeability and production of lactic acid as an indicator of carbohydrate metabolism.

In addition to the dose-dependent toxicity of different mine, coal and other mineral dusts, we studied the influence of cell culture media and the supplement of fetal calf serum (FCS) and bovine serum albumin (BSA) upon the cell toxicity in this test system.

We demonstrated a protective effect of FCS and BSA on dusts of low and medium toxicity, while dusts of high toxicity, like DQ_{12}, were not influenced in their toxicity.

Introduction

Lung macrophages play an important role in the pathogenesis of inflammation (*1*) and the development of fibrosis (*2*) after the inhalation of dusts. The exact mechanism of diseases caused by mixed dusts like mine dusts is still obscure. It seems to be clear that quartz and other forms of crystalline silica are significant in the evolution of pneumoconiosis. However, Seemayer et al. (*3*) demonstrated that quartz alone, in the mass concentrations usually found in coal mine dusts, exerts no detectable cytotoxic effects on guinea pig alveolar macrophages *in vitro*. Furthermore, Schlipköter et al. (*4*) demonstrated a protective effect of anthracite against the cytotoxic potential of quartz in the *in vitro* system. In the past, differing results were obtained using macrophages from different sources (*3, 5-11*) and diverse parameters and culture conditions.

Material and Methods

Cell Cultures of Alveolar Macrophages

Unstimulated guinea pig and rat lung macrophages were obtained by pulmonary lavage with heparinized (8 U/mL) Ca^{2+}- and Mg^{2+}- free phosphate buffered saline (PBS) (*5*). The resulting cell suspension was washed three times in heparin-free PBS, adjusted to 1.0×10^6 cells/mL and transferred into the cavities of multiwell plates (Falcon-Plastics No. 3008) in Minimum Essential Medium (MEM). After 2 hr, after cell attachment at 37°C in 5% CO_2/95% air, the MEM was removed, and the dusts were applied in different media with and without fetal calf serum (FCS) and bovine serum albumin (BSA) in different concentrations.

Lactic dehydrogenase and lactic acid were determined as described elsewhere (*12*).

Coal Mine and Control Dusts

Coal mine dust samples of four different coal mines in North-Rhine-Westfalia (NRW) and two dusts from the Sarre (S) mining area of FRG were tested. The physicochemical data of these dusts are listed in Table 1 (*13*). Dörentruper quartz (DQ_{12}) and electrocorundum with similar size distributions served as control dusts.

After weighing and sterilizing all dusts, samples were suspended in the suitable medium by ultrasound and applied in a concentration of 100 $\mu g/10^6$ cells after cell attachment.

Results and Discussion

Table 2 shows lactic dehydrogenase (LDH) release and lactic acid production of guinea pig lung macrophages 20 hr after exposure to 100 μg dust/10^6 cells in the presence of 5% FCS, 0.25% BSA and without any supplement.

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Table 1. Results of infrared spectroscopy and X-ray fluorescence analysis.

Dust	Quartz, %	Ash (380°C), %	Mineral content, %	Illite muscovite, %	Kaolinite, %	Montmorillonite, %	Volatile components, %	Specific surface, mg^2/g-%	Origin[a]
Y220	11.3	84.7	86.7	33.9	14.4	12.7	36.5	4.58	S
C120	7.6	56.9	56.5	19.9	16.5	5.7	35.8	3.58	NRW
V122	3.1	36.3	35.2	17.8	4.7	5.1	31.5	3.86	NRW
O120	17.3	71.5	74.1	30.7	10.7	7.2	37.1	4.33	S
N320	8.7	70.8	72.6	35.4	11.3	8.5	34.1	4.28	NRW
V220	9.0	66.3	70.3	31.2	6.0	6.0	34.3	4.16	NRW
H621	1.2	10.5	12.4	5.8	0.8	0.7	24.8	4.92	NRW
H520	1.0	8.5	8.6	4.6	0.3	0.6	19.2	4.29	NRW

[a]NRW = North Rhine-Westphalia; S = Sarre.

Table 2. LDH release and lactic acid production of guinea pig lung macrophages after incubation with 100 μg dust/10^6 cells under various culture conditions.

	LDH, mμ/10^6 cells			Lactic acid, mg/100mL		
Dust	Without serum	5% FCS	0.25% BSA	Without serum	5% FCS	0.25% BSA
Ko	3.3	0	4.6	10.2	9.9	11.0
Krd	5.8	0	13.4	9.7	14.2	13.5
DQ_{12}	83.3	76.4	89.2	3.6	4.7	3.6
Y220	60.5	0	14.8	11.2	11.2	13.5
C120	44.9	0	10.8	9.3	14.0	15.3
V122	38.7	0	8.0	10.6	10.4	12.1
O120	50.0	0	15.5	9.6	10.9	14.4
N320	48.5	0	20.4	12.7	12.0	16.6
V220	37.9	0	16.6	17.0	12.1	13.7
H621	6.8	0	7.2	21.2	17.6	17.8
H520	4.4	0	4.7	22.4	36.5	18.5

Table 3. Lactic dehydrogenase release and lactic acid production of guinea pig macrophages 20 and 44 hr after incubation with 100 μg dust/10^6 cells.

	20 hr, without FCS		20 hr, with 5% FCS		44 hr, with 5% FCS	
	LDH, mμ/10^6 cells	Lactic acid, mg/100mL	LDH mU/10^6 cells	Lactic acid, mg/100mL	LDH, mU/10^6 cells	Lactic acid, mg/100mL
Ko	7.1	14.0	2.6	16.2	9.7	23.0
Krd	8.5	12.7	1.0	16.1	5.4	29.4
DQ_{12}	96.2	2.8	78.4	2.7	80.6	8.2
Y220	64.6	12.7	3.5	14.3	13.4	30.3
C120	52.2	11.1	0	9.6	9.2	33.0
V122	44.2	12.5	4.8	10.7	13.9	25.4
O120	58.3	10.2	4.1	9.6	12.4	60.4
N320	50.5	15.4	3.1	10.3	12.9	29.8
V220	31.4	20.1	1.2	11.6	5.2	26.3
H621	10.2	16.2	5.2	13.0	12.7	32.1
H520	8.9	19.5	5.9	13.0	6.7	27.5

After incubation without addition of FCS of BSA, the results demonstrate a wide spectrum of toxicity in comparison to electrocorundum and DQ_{12}, as measured by LDH release. No correlation between quartz content and cytotoxicity can be observed. In the case of lactic acid production, no great differences can be observed. In two samples which are not toxic with regard to LDH release, the lactic acid production is even enhanced in comparison to the corundum control.

In the presence of 5% FCS and 0.25% BSA, only two of the six dusts which are toxic in the absence of any supplement exhibit a slight toxicity, i.e., increased LDH release when cultured with 0.25% BSA. Lactic acid production is not influenced by any of these dust samples, while the two nontoxic samples even enhanced carbohydrate metabolism.

The influence of incubation time under these standard conditions is documented in Table 3. It demonstrates that an incubation time of 44 hr in the

presence of FCS is not sufficient for the cells to abolish the protective affect of macromolecules in comparison to the exposure without FCS. When decreasing the concentration of FCS to 3.2 or 1% only dust V220 exhibits an increased toxicity (Fig. 1).

In further experiments we tested the influence of dust concentration. At the higher concentrations of 200 and 300 $\mu g/10^6$ cells in the presence of 1% serum, there is as slight increase of toxicity with respect to LDH (Fig. 2) and lactic acid (Fig. 3).

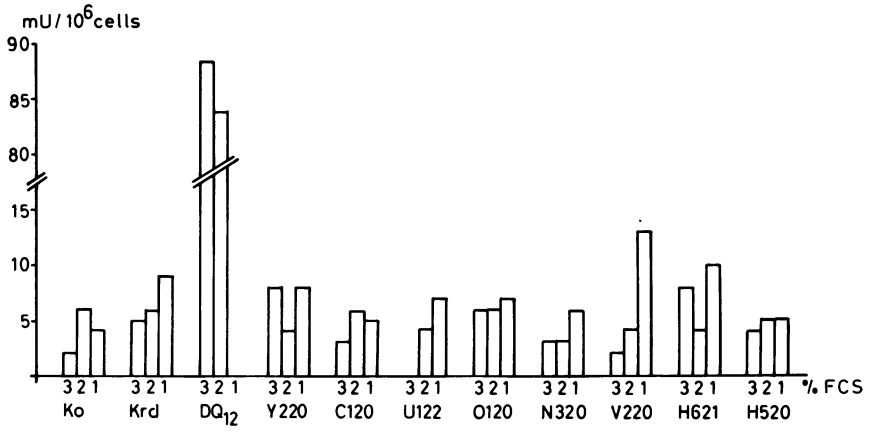

FIGURE 1. LDH release of guinea pig macrophages 20 hr after incubation with 100 $\mu g/10^6$ cells in the presence of 3, 2 and 1% FCS.

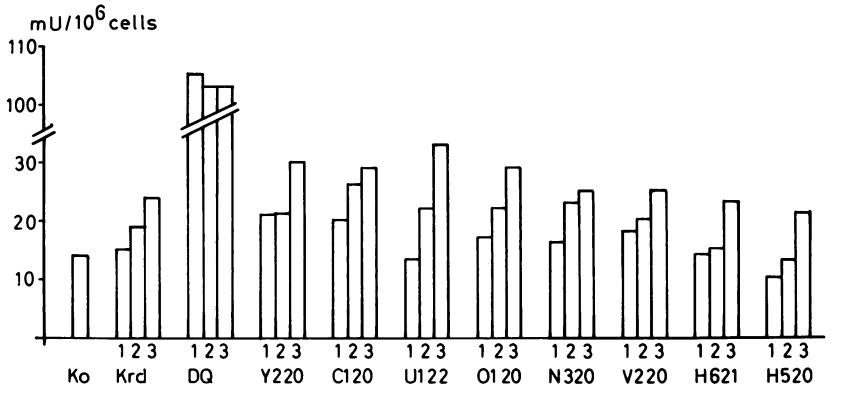

FIGURE 2. LDH release of guinea pig lung macrophages 20 hr after incubation with (1) 100, (2) 200 or (3) 300 μg dust/10^6 cells in the presence of 1% FCS.

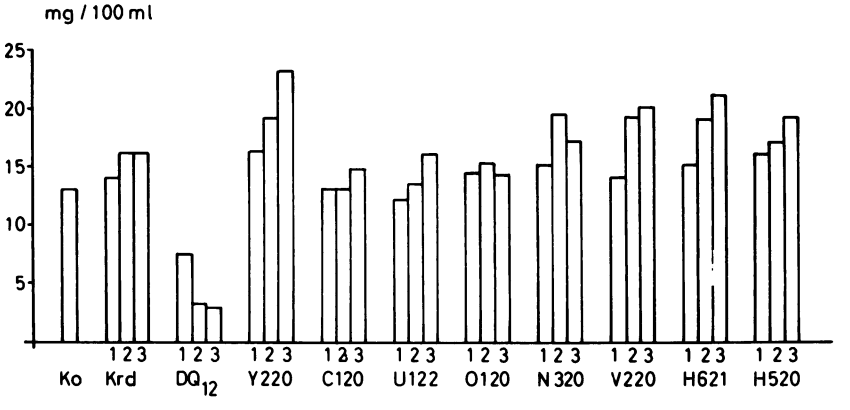

FIGURE 3. Lactic acid production of guinea pig macrophages 20 hr after incubation with (1) 100, (2) 200 or (3) 300 μg dust/10^6 cells in the presence of 1% FCS.

In further investigations we tested lower concentrations of FCS and BSA, and, with dusts showing a significant toxicity in serum-free medium, we found a FCS and BSA dose-dependent protective effect (Figs. 4 and 5). To test the possibility that macrophages are able to abolish this protective effect, we incubated rat lung macrophages in the presence of three different dosages of FCS over a period of 40 hr (Fig. 6). The protective effect of 0.5 and 1.0% FCS persists over this time period.

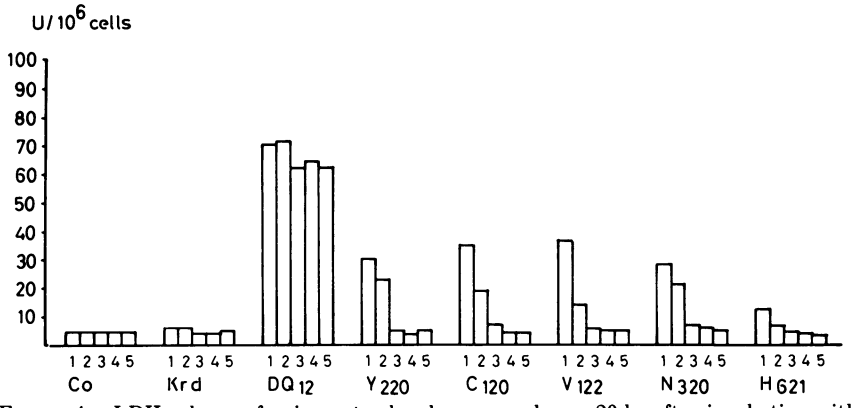

FIGURE 4. LDH release of guinea pig alveolar macrophages 20 hr after incubation with 100 μg dust/10^6 cells in the presence of various FCS concentrations: (1) 0%; (2) 0.0025% (3) 0.25%; (4) 0.05%; (5) 0.1%.

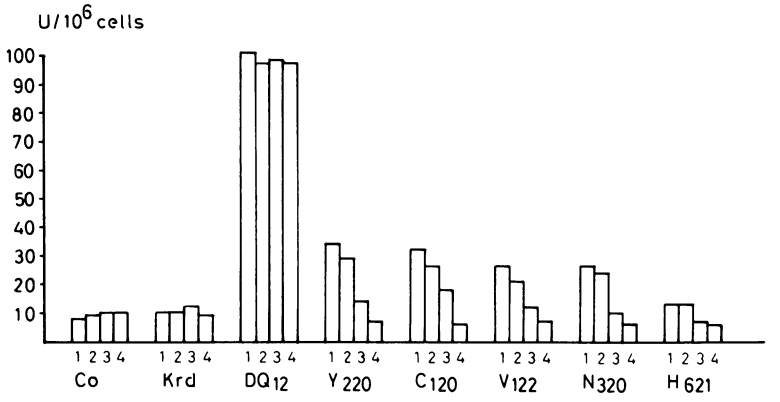

FIGURE 5. LDH release of guinea pig alveolar macrophages 20 hr after incubation with 100 μg dust/10^6 cells in the presence of various BSA concentrations: (1) 0%; (2) 0.0005%; (3) 0.005%; (4) 0.10%.

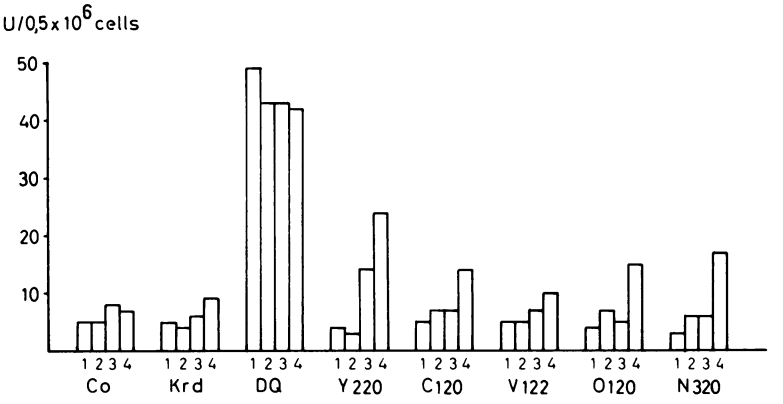

FIGURE 6. LDH release of rat alveolar macrophages 40 hr after incubation with 100 μg dust/10^6 cells in the presence of various FCS concentrations: (1) 1.0%; (2) 0.5%; (3) 0.1%; (4) 0%.

The results show that small quantities of protein molecules, like serum or BSA, have a considerable protective effect on the toxicity of coal mine dusts, while the toxicity of the control dust DQ_{12} is not or only slightly influenced by these protein concentrations; with serum concentrations of 30-40%, however (not demonstrated here), a significant decrease of DQ_{12} toxicity can be observed. Under these circumstances it appears important to use protein-free medium for the testing of coal mine dusts when employing long incubation times.

Earlier investigations (*12*) have demonstrated that the presence of Bactopepton with a molecular weight between 900 and 9000 in McCoy's 5 A Medium (Seromed, Munich, FRG) does not influence the toxicity of coal mine dusts in comparison to Minimum Essential Medium (MEM) and Medium NCTC 109. The fact that silica, such as DQ_{12}, is not influenced by proteins may be attributed to the effect of the large free silica surface in contrast to the natural coal mine dusts.

In the future it may be possible that the interpretation of the influence of proteins with different molecular weights on the toxicity of coal mine dusts gives further information about free silica or other toxic surfaces. In this context, the possible alteration of the surface in the course of dust preparation, e.g., the effect of sterilization and ultrasound treatment, should also be discussed and investigated.

REFERENCES

1. Schorlemmer, H. U., Ferluga, J., and Allison, A. C. Interactions of macrophages and complement components in the pathogenesis of chronic inflammation. In: Perspectives in Inflammation (D. A. Willonghby, J. P. Giroud and G. P. Vels, Eds.), Proc. 3rd Internatl. Meeting on Perspectives in Inflammation, MTP Press Ltd., 1977, pp. 191-206.
2. Heppleston, A. G. Biological response to silica. In: Biology of Fibroblasts (E. Kulonen and J. Pikkarainen, Eds.), Academic Press, London-New York, 1973 pp. 529-537.
3. Seemayer, N. H., Manojlovic, N., and deRuiter, N. Untersuchungen über die biologische Wirkung von Grubenstäuben. I. Zytotoxische Wirkung von Grubenstäuben auf alveolare Macrophagen des Meerschweinchens in-vitro. Silikosebericht Nordrhein-Westfalen 12: 173-180 (1979).
4. Schlipköter, H. W., Seemayer, N. H., and Manojlovic, N. Protektiver Effekt von Kohlestaub gegenüber der zytotoxischen Wirkung von Quarz in Makrophagenkulturen des Meerschweinchens. Bertr. Silikose Forsch. 28: 30-39 (1976).
5. Beck, E. G. Die Reaktion *in vitro* gezüchteter Zellen auf partikelförmige Verunreinigungen und hochpolymere Stoffe (Forschungsbericht NRW No. 2083), Westdeutscher Verlag, Köln Opladen, 1970, pp. 1-125.
6. Daniel, H., and Le Bouffant, L. Study of a quantitative scale for assessing the cytotoxicity of mineral dusts. In: The *In Vitro* Effects of Mineral Dusts (R. C. Brown, M. Chamberlain, R. Davies, and I. P. Gormley, Eds.), Academic Press, London-New York, 1980, pp. 33-39.
7. Robock, K., Beck, E. G., and Manojlovic, N. Die Sofortreaktion von Makrophagen auf SiO_2 -Staübe in vitro. Silikosebericht Nordrhein-Westfalen 9: 91-98 (1973)
8. Seemayer, N. H., and Manojlovic, N. Untersuchungen über die biologische Wirkung von Grubenstauben, II. Vergleichende Prüfung der Zytotoxizität von 16 verschiedenen Grubenstäuben an alveolaren Makrophagen des Meerschweinchens in vitro. Silikosebericht Nordrhein-Westfalen 12: 173-180 (1979).
9. Seemayer, N. H., and Manojlovic, N. Biological effects of coal mine dusts on macrophages. In: The *In Vitro* Effects of Mineral Dusts (R. C. Brown, M. Chamberlain, R. Davies and I. P. Gormley, Eds.), Academic Press, London-New York, 1980, pp. 5-12.
10. Seemayer, N. H. and Manojlovic, N. Untersuchungen zur spezifischen Schädlichkeit von Feinstäuben des Steinkohlenbergbaus, VI. Vergleich der Zelltoxizitat von 20 Grubenstauben an alveolaren Makrophagen in vitro. Silikosebericht Nordrhein-Westfalen 13: 225-232 (1981).
11. Tilkes, F., and Beck, E. G. Comparison of length-dependent cytotoxicity of inhalable asbestos and manmade mineral fibers. In: Biological Effects of Mineral Fibers, International Agency for Research on Cancer, World Health Organization, Lyon, 1980, p. 475.
12. Tilkes, F., and Beck, E. G. Macrophage functions after exposure to mineral fibers. Environ. Health Perspect. 51: 67-72 (1983).
13. Reisner, M. T. R., Frigge, J. Gnade, M., Schliephake, R. W., and Wolf-Fischer, E. Untersuchungen zur spezifischen Schädlichkeit von Feinstäuben des Steinkohlebergbaus. I. Analysen von Feinstäuben und Kohleproben. Silikosebericht Nordrhein-Westfalen 13: 171-185 (1981).